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doi: 10.1016/B978-0-444-59489-1.00002-1

Overview of Orexin/Hypocretin system

Michihiro Mieda and Takeshi Sakurai*

Department of Molecular Neuroscience and Integrative Physiology, Faculty of Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan.

*To whom correspondence should be addressed at:

Department of Molecular Neuroscience and Integrative Physiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Ishikawa 920-8640, Japan

Phone: +81-76-265-2173, FAX: +81-76-234-4224

Email: tsakurai@med.kanazawa-u.ac.jp

Abstract

A series of recent studies has established the orexin/hypocretin system as a critical regulator of sleep/wake states. Its deficiency results in the sleep disorder narcolepsy in humans, dogs, and rodents. These findings have brought about the possibility of novel therapies for sleep disorders including narcolepsy and insomnia. Moreover, accumulating evidence indicates that the orexin/hypocretin system regulates sleep and wakefulness through interactions with neuronal systems that regulate emotion, reward, and energy homeostasis. Here, we briefly summarize the progress of orexin/hypocretin studies and future perspectives.

Key words

Orexin/hypocretin, sleep/wakefulness, narcolepsy, feeding, reward

Introduction

Since its discovery in 1998, the field of orexin/hypocretin biology is continuing to expand. Discovery of orexin/hypocretin peptides led to elucidation of an unexpected causal relationship between deficiency of the orexin/hypocretin system and the human sleep disorder narcolepsy, which brought about a huge impact on the study of sleep and wakefulness. Subsequent studies further expanded orexin/hypocretin biology beyond the field of sleep/wakefulness, shedding light on the integrative role of the orexin/hypocretin system. In parallel, attempts to apply orexin/hypocretin biology to clinical areas have led to the development of a novel and definite diagnosis of narcolepsy, and are further making progress towards novel treatments for sleep-related disorders.

Because details of every aspect of orexin/hypocretin biology will be discussed in the following chapters, we will here overview the lines of research on orexin/hypocretin biology, and further discuss its still unanswered questions and future directions.

Discovery of orexin/hypocretin

Completely different approaches taken by two independent laboratories beautifully converged on the identification of orexin/hypocretin peptides in 1998. de Lecea et al. utilized molecular biological techniques (de Lecea et al., 1998). They had previously isolated a series of cDNA clones that are expressed in the hypothalamus but not in the cerebellum and the hippocampus by subtractive hybridization. One of these was expressed exclusively by a bilaterally symmetric structure within the posterior lateral

hypothalamus. They subsequently cloned cDNAs covering the entire coding region, which encodes a putative secretory protein of 130 amino acids. According to its primary sequence, this protein was predicted to produce two peptide products that are structurally related to each other. Since these predicted peptides were expressed in the hypothalamus and the authors thought these peptides had similarity to secretin, they named them hypocretin-1 and -2, although orexins/hypocretins later turned out not to be relatives of the incretin family. Antibodies raised against prepro-hypocretin identified hypocretin-positive cell bodies exclusively in the perifornical area of the hypothalamus and hypocretin-positive nerve fibers in many brain areas. It was further demonstrated that hypocretin is in synaptic vesicles by electron microscopy, and that hypocretin-2 has excitatory effects on hypothalamic neurons by electrophysiological study. Thus, their study suggested that hypocretin peptides are novel neurotransmitters exclusively expressed in a population of neurons in the perifornical area.

Around the same time as the report by de Lecea et al., Sakurai et al. reported identification of novel neuropeptides, termed orexin-A and orexin-B, as endogenous ligands of two orphan G-protein coupled receptors (GPCRs whose cognate ligands have not been identified, named orexin receptor 1: OX1R and orexin receptor 2: OX2R) (Sakurai et al., 1998). These peptides are cleaved from a common single precursor polypeptide, prepro-orexin, and are expressed by a particular population of neurons clustered around the lateral hypothalamus. It turned out later that prepro-orexin is identical to prepro-hypocretin and that orexin-A and -B correspond to hypocretin-1 and -2, respectively, although the original predicted structures of hypocretin-1 and -2 were not identical to purified orexin-A and -B because of incorrect prediction of proteolytic sites, as well as loss of two intrachain disulfate bonds and N-terminal pyroglutamylation

in hypocretin-1, which were found in orexin-A.

Since these peptides were exclusively expressed in the lateral hypothalamus, which has been known as the "feeding center", intracerebroventricular (ICV) administration of orexin-A or -B was performed to observe the action of these peptides on feeding behavior. Orexins are found to increase food intake in rats in a dose-dependent manner; this was the reason these peptides were named "orexin" after the Greek word orexis, which means appetite. Furthermore, like other mRNAs encoding orexigenic peptides such as neuropeptide Y, expression of prepro-orexin mRNA was up-regulated more than two-fold upon fasting.

Loss of orexin signaling causes narcolepsy

Soon after the discovery of orexin, two independent studies utilizing dog forward genetics and mouse reverse genetics unveiled the causal relationship between orexin deficiency and narcolepsy.

Human narcolepsy is a debilitating neurological disease characterized by excessive daytime sleepiness (an insurmountable urge to sleep), which often results in falling asleep at inappropriate times and situations ("sleep attacks"), premature transitions to REM sleep (so called "sleep-onset REM periods"), and cataplexy (sudden bilateral skeletal muscle weakness without impairment of consciousness) (Bassetti and Aldrich, 1996). A Stanford University group has established and maintained canine breeds with autosomal recessive inheritance of a narcolepsy syndrome for decades. In 1999, Lin et al. identified mutations in the OX2R gene responsible for canine narcolepsy by positional cloning (Lin et al., 1999).

Around the same time, Chemelli et al. reported that prepro-orexin knockout

mice exhibit a phenotype strikingly similar to human narcolepsy, characterized by cataplexy-like abrupt behavioral arrests, fragmentation of wakefulness and non-REM sleep, characterized by very short wakefulness and NREM sleep episode durations, and direct transitions from wakefulness to REM sleep (Chemelli et al., 1999). Consistent with a presumed critical role of orexin in sleep/wake regulation, orexin-immunoreactive nerve terminals were observed on neurons implicated in arousal regulation, such as the locus coeruleus (LC) noradrenergic neurons, raphe serotonergic neurons, tuberomammillary nucleus (TMN) histaminergic neurons, and pedunculopontine tegmental nucleus/laterodorsal tegmental nucleus (PPT/LDT) and basal forebrain cholinergic neurons. Additionally, orexin receptor subtypes are expressed in these regions with different expression patterns (Marcus et al., 2001; Mieda et al., 2011).

Subsequently, deficiency of orexin neurons in human narcolepsy was confirmed and reported by three laboratories in 2000. Nishino et al. found that orexin-A was undetectable in the cerebrospinal fluid (CSF) of seven out of nine narcolepsy patients, but readily detected in normal control individuals (Nishino et al., 2000). Peyron et al. and Thannickal et al. further reported marked reductions of orexin mRNA and immunoreactivity in postmortem brains of narcolepsy patients (Peyron et al., 2000; Thannickal et al., 2000). Furthermore, an unusually severe, early onset case of human narcolepsy was associated with a mutation in the orexin gene that impairs peptide trafficking and processing (Peyron et al., 2000).

These studies of humans and animals collectively established that failure of signaling mediated by orexin neuropeptides causes narcolepsy.

Orexin neurons as stabilizer of sleep/wakefulness states

Even before the discovery of linkage between orexin and narcolepsy, anatomical and pharmacological studies had suggested the involvement of these peptides in sleep/wakefulness regulation (Peyron et al., 1998; Hagan et al., 1999). After this epoch-making discovery, a whole series of studies confirmed that the orexin system plays a central role in the regulation of sleep/wakefulness. Neurons expressing orexin (orexin neurons) activate nuclei considered wake-promoting, including LC noradrenergic neurons (Horvath et al., 1999; van den Pol et al., 2002), raphe serotonergic neurons (Brown et al., 2001; Liu et al., 2002), TMN histaminergic neurons (Bayer et al., 2001; Yamanaka et al., 2002), and PPT/LDT and basal forebrain cholinergic neurons (Eggermann et al., 2001; Eriksson et al., 2001). Central administration of orexin-A in rodents reduces REM and non-REM sleep, and increases wakefulness time (Hagan et al., 1999).

Microinjections of orexin directly into the LC (Bourgin et al., 2000), TMN (Huang et al., 2001), BF cholinergic area (Espana et al., 2001), LDT (Xi et al., 2001), and lateral preoptic area (Methippara et al., 2000) has arousal effects similar to those of ICV injection on sleep/wakefulness states. Recent optogenetic and pharmacogenetic manipulations of orexin neurons established a definite causal relation between their neuronal activities and transitions of sleep/wakefulness states (Adamantidis et al., 2007; Sasaki et al., 2011; Tsunematsu et al., 2011).

Although these data suggest that activation of orexin neurons is sufficient to promote and maintain wakefulness, their roles in the transitions between wakefulness and sleep in natural, physiological conditions still remain unclear.

In vivo single unit recordings by three laboratories revealed activity patterns

of orexin neurons across sleep/wakefulness cycles in rats and mice with high temporal resolution (Lee et al., 2005; Mileykovskiy et al., 2005; Takahashi et al., 2008). Essentially, orexin neurons fired most actively during active waking, showed decreased discharge during quiet waking, were virtually silent during NREM sleep, and were almost silent but exhibited occasional firing during REM sleep. During the transition from sleep to wakefulness, orexin neurons fired prior to the onset of EEG activation. However, it remains unknown whether orexin neurons initiate awake episodes, or other wakefulness-promoting neurons activate orexin neurons. Considering the facts that narcoleptics can be roused from sleep and their daily amount of wakefulness is relatively similar to that in normal controls, the latter case may be more likely.

On the other hand, the fact that orexin neurons are much more active during active wakefulness than during quiet wakefulness *in vivo* clearly suggests that the roles of orexin extend beyond mere global arousal (Lee et al., 2005; Mileykovskiy et al., 2005). Consistently, a recent study implicated orexin in central vestibular motor control (Zhang et al., 2011). Further examination of context-dependent firing patterns of orexin neurons is needed.

Narcolepsy is characterized by the inability to maintain wakefulness states, pathological intrusion of REM sleep into wakefulness, and frequent transitions between states of sleep and wakefulness (Bassetti and Aldrich, 1996), which suggests that orexins inhibit inappropriate transition between each vigilance state. This action could play important roles in the maintenance and stabilization of sleep and wakefulness.

The sleep/wake cycle is thought to be regulated by the balance between the sleep-center (sleep-active neurons in the ventrolateral preoptic area have been suggested as its correlate) and wake-center (wake-active monoaminergic neurons in the

hypothalamus and brain stem have been suggested as its correlate), which reciprocally inhibit each other constituting a “flip-flop” circuit (Saper et al., 2001; Sakurai, 2007). In this type of circuit, when activity on either side begins to overcome the other, the system will flip to one of two possible extremes. Although it is well suited to avoiding intermediate states, a small perturbation of the activity on one side can easily cause abrupt switching between the two states, resulting in frequent state transitions. Such a condition resembles the narcoleptic phenotype. Orexin neurons are likely to function as a stabilizer of this circuit by enhancing the activity of monoaminergic neurons during wakefulness on demand, avoiding state instability caused by small perturbations (Sakurai, 2007).

Integrative physiology of orexin system

In addition to sleep/wakefulness regulation, early descriptions of the projection patterns of orexin neurons had already suggested their involvement in a wide range of other physiological functions, such as feeding, autonomic regulation, and neuroendocrine regulation (Peyron et al., 1998; Date et al., 1999). Consistently, central administration of orexin causes a wide variety of effects (Sakurai, 2007).

The physiological implications of the orexin system are still continuing to spread, placing orexin neurons as a link between the arousal center and many other systems.

As mentioned above, orexins were initially characterized as orexigenic peptides (Sakurai et al., 1998). An important difference in the effects on feeding between orexin and other orexigenic factors, such as NPY and melanin-concentrating hormone (MCH), is that orexin increases both food intake and energy expenditure

(Lubkin and Stricker-Krongrad, 1998; Hara et al., 2001), while other feeding peptides generally decrease energy expenditure (Spiegelman and Flier, 2001): the latter response is more adaptive to conserving energy under food scarcity. Increased energy expenditure by orexin administration seems to be caused by increased wakefulness and locomotor activity, as well as an increase in sympathetic outflow. Indeed, orexin deficiency decreases sympathetic tone (Kayaba et al., 2003; Zhang et al., 2006), resulting in reduced energy expenditure. This may explain why human and mouse narcolepsy are associated with an increase of body weight despite hypophagia (Lammers et al., 1996; Schuld et al., 2000; Hara et al., 2001). Additionally, a recent work suggested that orexin enhances leptin-sensitivity through an OX2R-mediated mechanism (Funato et al., 2009).

Thus, orexin neurons do not simply act as a system that maintains long-term body weight homeostasis. Rather, they seem to be necessary for food seeking and feeding behavior, especially when animals are faced with food scarcity. Food seeking and food intake require vigilant states which require high energy expenditure. Recent evidence suggests that orexin neurons are capable of sensing indicators of energy balance and are activated under negative energy balance, such as decreased extracellular glucose level, reduced leptin level (an anorexigenic protein hormone secreted by adipocytes), and increased ghrelin level (a stomach-derived orexigenic peptide) (Yamanaka et al., 2003; Burdakov et al., 2005). Recently, non-essential amino acids were also reported to activate orexin neurons, which may potentially benefit an animal under prolonged starvation where a rise in extracellular amino acid levels occurs as proteins are broken down for fuel (Karnani et al., 2011).

When faced with a negative energy balance due to reduced food availability,

mammals respond behaviorally with phases of increased wakefulness and alertness, which presumably enhances their ability to find food. We previously demonstrated that orexin neuron-ablated mice are incapable of this fasting-induced arousal, indicating that orexin neurons are necessary for evoking adaptive behavioral arousal during fasting (Yamanaka et al., 2003). Coordinated increases of sympathetic and hypothalamic-pituitary-adrenal (HPA) tone in response to fasting-induced arousal directed by orexin neurons may further help animals to execute adaptive behavior.

Orexin neurons are likely to promote arousal and regulate autonomic and endocrine systems according also to water balance, by responding to the antidiuretic hormone arginine vasopressin (Tsunematsu et al., 2008), and acid-base homeostasis, by sensing changes in pH directly (Williams et al., 2007).

In this context, emotional states may be another factor through which orexin neurons sense and regulate the internal environment accordingly to wake up and execute behaviors. Especially under salient conditions, animals exhibit increased arousal and vigilance levels, accompanied by increased sympathetic outflow and HPA axis activity. Orexin neurons are likely to be involved in the coordinated regulation of these responses in stressful environments (Kayaba et al., 2003; Winsky-Sommerer et al., 2004; Zhang et al., 2006). Furthermore, the orexin system has been also implicated in analgesia. Especially, the corticotropin-releasing factor (CRF) and nociceptin/orphanin FQ (N/OFQ) systems modulate orexin neurons in a coordinated manner to regulate stress-induced analgesia, a key component of the defensive behavioral “fight or flight” response (Xie et al., 2008).

The roles of orexin neurons in the reward system have been a focus of recent attention. Dopaminergic projections of neurons in the midbrain ventral tegmental area

(VTA) to the forebrain, particularly to the nucleus accumbens (NAc), have classically been identified as the “reward pathway”. Drugs of abuse stimulate this pathway, and orexin neurons have reciprocal connections with both the VTA and NAc. Orexin signaling has been reported to be critical for morphine-induced place preference and hyperlocomotion, and reinstatement of extinguished drug-seeking behavior in mice and rats (Harris et al., 2005; Narita et al., 2006). Notably, narcolepsy patients with daytime sleepiness who were treated with amphetamine-like stimulants and/or GHB for a long time rarely developed drug addiction (Guilleminault et al., 1974). These observations indicate a strong functional interaction between the orexinergic pathways and the dopaminergic system in the mechanisms of reward and drug addiction. This interaction may underlie the hedonic control of feeding, as well as the facilitating role of orexin in male sexual behavior of rats (Muschamp et al., 2007).

On the other hand, Boutrel *et al*. reported that orexin-A/hypocretin-1 reinstates cocaine seeking by mechanisms different from increased dopamine release, rather through induction of a stress-like state (Boutrel et al., 2005). Thus, precise consideration of the interactions among the orexin, reward, and stress systems would be important. Nevertheless, these findings highlight the key role of orexin in the mechanisms of reward and drug addiction.

Thus, the reciprocal interactions between orexin neurons and multiple neuronal systems raise the possibility that orexin neurons function as an interface between multiple regulatory systems including feeding, reward, emotional, autonomic, and endocrine systems (Fig. 1). This notion is further supported by recent systematic searches of afferent pathways to orexin neurons (Sakurai et al., 2005; Yoshida et al., 2006). Understanding the connectome of orexin neurons more precisely might provide

further insights into how the systems regulating emotion, energy homeostasis and reward interact with the mechanism that regulates sleep and wakefulness. Further precise mechanisms of actions of these peptides could be elucidated by studies dissecting the roles of each orexin receptor in particular brain regions, with the aid of brain region-specific deletion of orexin receptor genes, as well as brain region-specific rescue of receptor expression in narcoleptic orexin receptor knockout mice. In addition, the detailed mechanisms underlying context-dependent regulation of orexin neurons would be revealed by analyses of mutant mice in which receptors for signaling molecules that regulate the activity of orexin neurons are deleted specifically in orexin neurons.

Clinical perspectives

The low CSF orexin-A level in patients with narcolepsy led to the development of a novel, definitive diagnostic test for this disease (Mignot et al., 2002). Currently, a low orexin-A level in CSF (less than 110 pg/ml) is one of the diagnostic criteria for narcolepsy according to the 2nd edition of the International Classification of Sleep Disorders (American Academy of Sleep Medicine, 2005).

In addition, the discovery of a causal link between loss of orexin signaling and human narcolepsy has brought about the possibility of novel therapies for this disease. Currently, excessive sleepiness is treated using psychostimulants, while cataplexy is treated with tricyclic antidepressants (Zeitzer et al., 2006). γ-hydroxybutyrate (sodium oxybate, GHB) is also used to consolidate nocturnal sleep and reduce cataplexy. This therapeutic regimen is problematic due to limited effectiveness, undesirable side effects such as insomnia or symptom rebound, and the

potential for abuse. We demonstrated that orexin neuron-ablated mice, the most accurate pathophysiological murine model of narcolepsy available (Hara et al., 2001), retain the ability to respond to ICV administration of orexin neuropeptides, with stabilization of wake episodes and prevention of cataleptic attacks (Mieda et al., 2004), suggesting that orexin receptor agonists would be of potential value for treating human narcolepsy.

Conversely, orexin receptor antagonists might be useful as safe hypnotics. Indeed, Almorexant (ACT078573, Actelion Pharmaceuticals Ltd.), an orally available antagonist of both receptors, has been reported to increase subjective and objective electrophysiological signs of sleep in humans (Brisbare-Roch et al., 2007).

Suvorexant (MK4305, Merck & Co., Inc.) is another compound with potent dual orexin receptor antagonistic activity (Cox et al., 2010). This compound is currently under Phase IIIb clinical trials for the treatment of primary insomnia. Recently, administration of an OX2R selective antagonist, JNJ10397049, in rats was reported to be more potent than the dual antagonist almorexant to decrease the latency for persistent sleep and increase NREM sleep time (Dugovic et al., 2009). Thus, selective OX2R antagonists may offer a possible advantage for the treatment of insomnia.

Orexin initially drew attention as a regulator of food intake. Therefore, several pharmaceutical companies developed orexin receptor antagonists to control appetite for obesity treatment. Indeed, an OX1R-selective antagonist, SB334867, reduced food intake and ameliorated obesity in leptin-deficient ob/ob mice (Haynes et al., 2002).

As described above, orexin mediates many behaviors associated with drug addiction in rodents owing to its effects on the VTA. A recent report showed that the orexin-1 receptor antagonist SB334867 reduces the acquisition and expression of cocaine-conditioned reinforcement and the expression of amphetamine-conditioned

reward, suggesting that OX1R antagonists have potential as a treatment for individuals struggling with drug relapse and dependency (Hutcheson et al., 2011), although whether the effects of orexin are the same in humans needs to be confirmed. This notion is supported by the fact that drug addiction is seldom found in narcolepsy patients who are treated with psychostimulants (Guilleminault et al., 1974).

OX1R antagonists might also be effective for panic disorders. They inhibit the increased mean arterial pressure, heart rate and freezing responses in rat models of panic disorder (Johnson et al., 2010).

We still do not know well why there is specific degeneration of orexin neurons in narcolepsy. Narcolepsy has been speculated to be an autoimmune disease because of its strong association with certain HLA alleles, which was revealed when the causal relationship of the orexin system with this disease was not known, suggesting that narcolepsy may result from selective autoimmune degeneration of orexin neurons (Kadotani et al., 1998). Recently, Tribbles homolog 2 (Trib2) was reported as a possible antigen involved in the autoimmune destruction of orexin neurons (Cvetkovic-Lopes et al., 2010). Recent studies also showed that susceptibility to narcolepsy is associated with SNPs in the *T-cell receptor alpha* locus (Hallmayer et al., 2009) and between the *carnitine palmitoyl-transferase 1B* and *choline kinase* β loci (Miyagawa et al., 2008). Understanding the molecular and genetic mechanisms underlying degeneration of orexin neurons in the development of narcolepsy would lead to accurate estimation of disease risk and further to prevention of disease onset.

Figure legend

Figure 1. Orexin neurons function as an interface between multiple regulatory systems including feeding, reward, emotional, autonomic, and endocrine systems. Orexin neurons in the lateral hypothalamic area (LHA) and posterior hypothalamus (PH) are anatomically well placed to provide a link between the limbic system, systems involved in energy homeostasis, and wakefulness-promoting monoaminergic neurons in the brain stem. Solid arrows show excitatory projections, and broken lines inhibitory projections. Orexin neurons promote wakefulness through monoaminergic nuclei that are wake-active. Stimulation of dopaminergic centers by orexins can modulate reward systems. Peripheral metabolic signals such as leptin, ghrelin and glucose influence orexin neuronal activity to coordinate arousal and energy homeostasis. The nucleus suprachiasmaticus (SCN), the central body clock, sends signals to orexin neurons via the dorsomedial hypothalamus (DMH). Input from the limbic system (amygdala and bed nucleus of the stria terminalis (BST)) might regulate the activity of orexin neurons upon presentation of emotional stimuli that evoke emotional arousal or fear-related responses. Orexin neurons also control sympathetic outflow and the neuroendocrine system according to the arousal state. VLPO, ventrolateral preoptic area; DR, dorsal raphe; GABA, γ-aminobutyric acid; LC, locus coeruleus; TMN, tuberomammillary nucleus.

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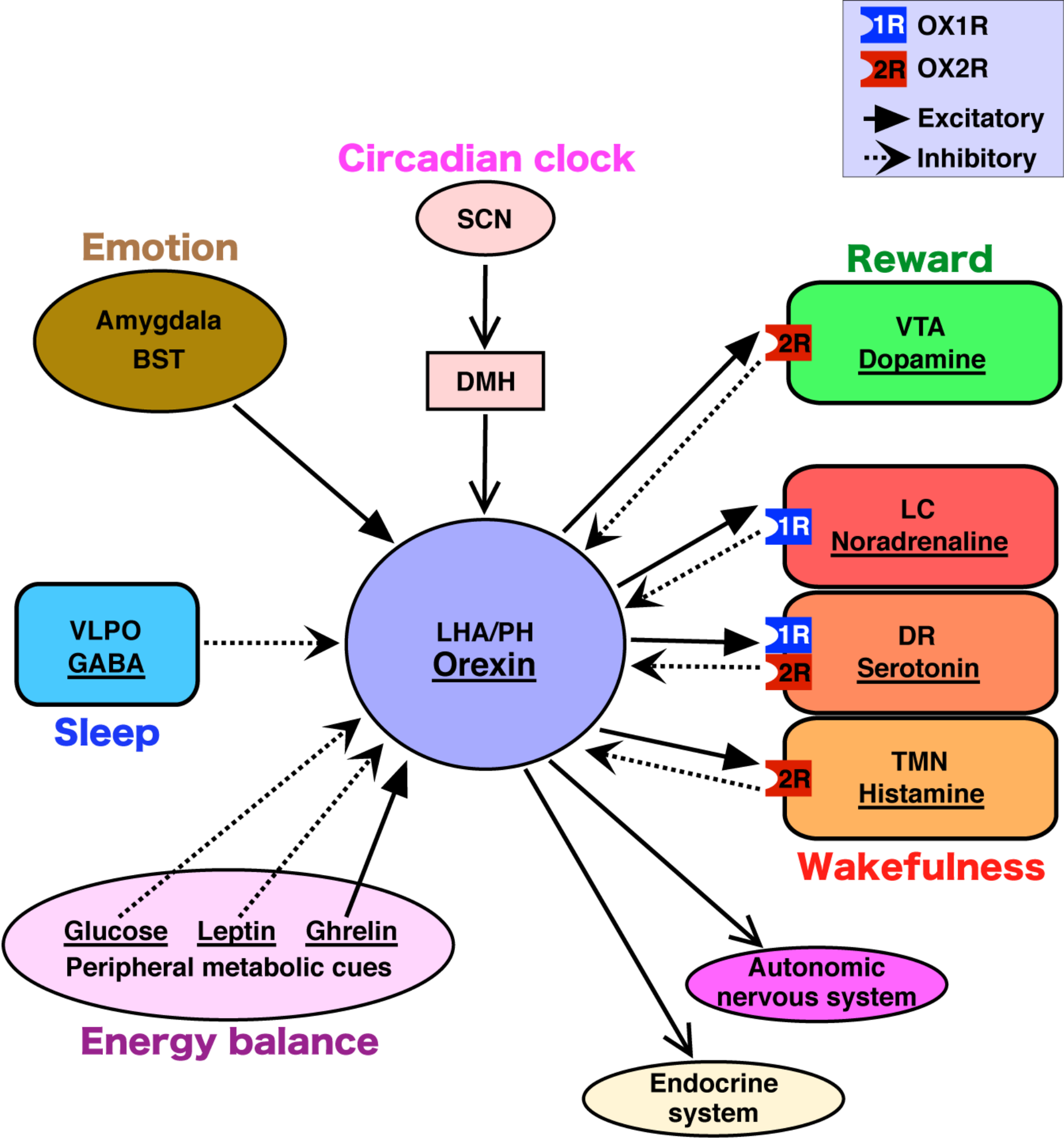
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1R
LC
Noradrenaline
VLPO
GABA
LHA/PH
Orexin
1R
2R
DR
Serotonin
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TMN
Histamine
Sleep
Wakefulness
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